Mycoplasma Testing of Cell Sorters – Standard Operating Procedure

Materials Required:

- Thermocycler (recommended) or heat block.
- Pipettes with corresponding filter tips (recommended)
- 0.2 mL and 1.5 mL microcentrifuge tubes
- Bench Centrifuge
- Sterile phosphate buffered saline (PBS)
- MycoStrip Detection Kit (InvivoGen)
    - Reaction Mix
    - Reaction Buffer
    - Migration Buffer
    - Positive Control
    - Detection Strips

Reagent Preparation:

- Allow all ***samples*** and ***reagents*** to come to room temperature.
- Upon first use: ***Reaction Mix***
    - Spin down and gently homogenize (by flicking) 1 vial of the Reaction Mix
    - Prepare 10x 15 uL aliquots, plus any extra.
    - Use aliquots immediately or store at -20°C until required.
- Mix the ***Reaction Buffer*** very well by several vortex pulses.

Sample Preparation:

Sheath Tanks:

- Fill a microcentrifuge tube with at least 1 mL of fluid from each sheath tank.
- Further testing sites: cell sorter streams, fluid containers/carboys, sheath tanks

Cell Supernatant:

- Transfer 1 mL of cell supernatant to a microcentrifuge tube.
- Centrifuge for 5 minutes at 16,000g.
- Using a pipette discard the supernatant (leave about 50 uL of supernatant)
- Add 500 uL of sterile PBS.
- Mix Well

Assay Procedure:

Key Notes:

- Remove kit from storage and allow reagents to warm to room temperature.
- Set Thermocycler or heat block to 65°C before starting assay.
- Include a negative control by using sterile PBS as a sample.
- DO NOT exceed 65°C and 40 minutes.

1. In a 15 uL Reaction Mix aliquot, add first:
    - 5 uL of the Reaction buffer, then
    - 5 uL of the prepared sample (or control), and gently mix, DO NOT VORTEX
2. Incubate the reaction tube at 65°C for 40 minutes using a thermocycler or heat block.
3. Remove samples from heat and immediately add 200 uL of Migration Buffer to stop the reaction, Mix well.
4. Place the detection strip directly into the processed sample with the conjugation pad (pink) end in the tube. Ensure the sample pad is submerged and wait for 2 to 5 minutes for results.

Interpretation of Results:

- There are two distinct results:
    - One Band ('C' band only): Indicates that mycoplasma was not detected in the processed sample.
    - Two bands ('C' and 'T' band): Indicates the processed sample is contaminated by mycoplasma.
- To confirm a 'faint" positive result: Concentrate your sample or continue to grow the culture for an additional 48 hours (about 2 days).

Sensitivity and Specificity:

MycoStrip is specifically designed to detect the mycoplasma and Acholeplasma species that most commonly contaminate cell cultures. These include six species that account for 95% of all contaminations.

1. *M. orale*
2. *M. hyorhinis*
3. *M. arginini*
4. *M. fermentans*
5. *M. hominis*
6. *A. laidlawii*

Results are published using the template below and updated to our website.

Mycoplasma Testing Results (MycoStrip – InvivoGen Lot: 10619-46-05)

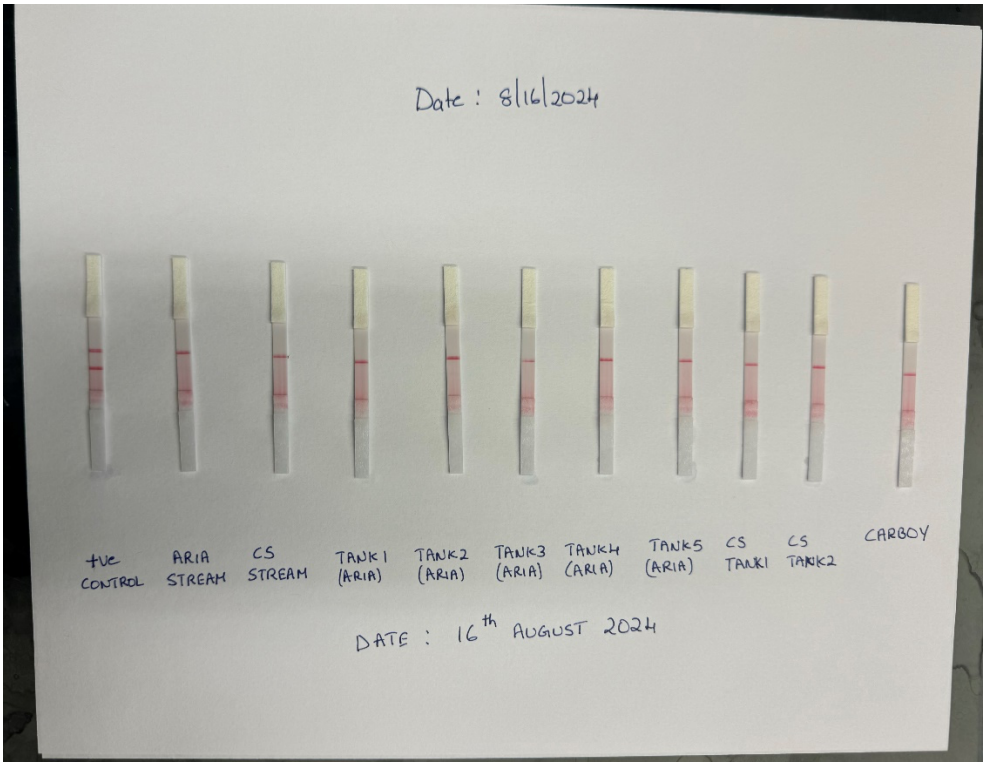
Date: 16th August 2024

Location	Band (C/and or T)	Results	Pass/Fail
Carboy 1	C	Negative	Pass
Aria Stream	C	Negative	Pass
Sheath Tank 1	C	Negative	Pass
Sheath Tank 2	C	Negative	Pass
Sheath Tank 3	C	Negative	Pass
Sheath Tank 4	C	Negative	Pass
Sheath Tank 5	C	Negative	Pass
CS Sheath Tank 1	C	Negative	Pass
CS Stream	C	Negative	Pass
Positive Control	C and T	Positive	Pass

Interpretation:

C Band: Mycoplasma Not Detected
T Band: Mycoplasma Detected

Results:



Remarks: None

Standard Operating Procedure			
Mycoplasma Testing SOP	**Approval Date**	**Approved By**	
	2/26/2024		
Revision History			
Revision Date	**Version #**	**Change**	**Sections**
2/26/24	1.00	Removed Header, added revision history	N/A
10/28/2024	1.01	Updated wording and added results reporting page.	